modulated/enhanced/expanded only through its interactions with heterotropic effectors: P_{50} from 1 to >100 $_{mmHg}$, K_R from 10 to 0.005 $_{/mmHg}$, K_T from 0.3 to 0.005 $_{/mmHg}$, K_R/K_T from 1 to >500, and ΔH^+ up to −4.4 H^+/Hb. Such enhanced functionality of Hb of physiological relevance is accomplished through pH-dependent asymmetric binding of heterotropic effectors to R(oxy)- and T(deoxy)-quaternary structures of Hb, which cause pH-dependent differential modulations/reductions of K_R and K_T, respectively. No changes in high-resolution static crystallographic T(deoxy)- and R(oxy)-quaternary and tertiary structures of Hb and their heme environment, as well as the axial coordination structures of the deoxy-heme ($\nu_{Fe\text{-}His}$ = 215 $_{/cm}$) and the oxy-heme ($\nu_{Fe\text{-}O2}$ = 567$_{/cm}$) in solution, are observed, despite K_T and K_R values are changed as much as 100- and 2,000-folds, respectively. Thus, the assumption that the low-affinity state is caused by the inter-dimeric salt-bridge-linked constraints, the out-of-plane shift of the heme Fe, and the allosteric core constraint in the T(deoxy)-Hb is no longer valid. Although these constraints are completely absent in R(oxy)-Hb, its O_2-affinity is modulated as much as 2,000-folds by its interaction with heterotropic effectors. The effector-linked modulation of thermal fluctuation of the protein may be responsible.

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Porphyrins as Detectors of Internal Electric Field in Heme Proteins

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Heme proteins display remarkable specificity in discriminating between diatomic ligands that are similar in structure, such as dioxygen, carbon monoxide, and nitric oxide. However, the factors affecting their ability to accomplish this are not known with certainty. We are exploring the influence of the internal electric field produced by the protein matrix on the binding affinities at its heme active site. Generally, the ''structure determines function'' paradigm is employed to formulate a steric mechanism. Here we present a unique ''electrostatic structure'' approach to this question, using myoglobin as a model protein. The porphyrin residing at the active site provides a spectroscopic report of the electrostatic environment. In a first step, the atoms comprising the protein matrix are treated as point charges, and their individual electric field contributions are calculated using Coulomb's Law and summed at the heme site. Point mutations surrounding the active site are introduced and the effect on the internal electric field value is noted. In addition, the fluctuation of the field due to natural breathing motions of the protein in solution is modeled, posing the possibility of alternating fields playing a role in ligand discrimination. Experimentally, hole-burning spectroscopy provides a way to extract internal electric field values. This technique uses the Stark effect which is an application of an external electric field perturb the energy of the porphyrin detector. Preliminary results are determined to be quite significant - on the order of megavolts per centimeter. Finally, an excited state analysis of our detector porphyrins using Gaussian09 is presented, including transition dipole moment and state energy values. These values play an important role in the interpretation of spectroscopic data.

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MD Simulations of Plant Hemoglobins: the Hexa- to Penta-Coordinate Structural Transition

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Hemoglobins are ubiquitous proteins found in bacteria, plants, and animals with diverse functions other than the classical transport/storage of oxygen. Different functions are expected to correspond to substantially different structures, such as the hexa- and penta-coordination of the iron atom. It is now widely believed that pentacoordinate hemoglobins evolved from the hexacoordinate ones, both in plants and in animals. Since plant hemoglobins evolved more recently than in animals, they represent a simpler and thus useful system to investigate protein sequence/structure features that specifically supported, guided by molecular evolution, the capacity for oxygen transport. In the present work, we selected a fully hexacoordinate globin, AHb2 from Arabidopsis thaliana and the pentacoordinate oxygen-transporting LegHb from yellow lupin, that share a high degree of sequence identity (80%). With the aim is to identify the structural determinants for oxygen transport we analyzed the structural/dynamical differences of a hexacoordinate and a pentacoordinate globin using all-atom molecular dynamics simulations in the microsecond time scale. Using comparative MD simulations, we were able to go beyond the simple sequence alignment, pointing out important differences between these two hemoglobins especially at the level of the CD region, whose dynamics was found, in turn, to be strongly correlated with that of the distal region.

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Impact of the Internal Disulfide Bond on Structural Dynamics in Vertebrates Hexacoordinate Hemeoglobins

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There are two new additions to the vertebrate globins family, neuroglobin and cytoglobin, which exhibit bis-hystidyl hexacoordination. Mainly expressed in neurons, neuroglobin (Ngb) has a neuro-protective role under hypoxic-ischemic insults; whereas cytoglobin (Cygb) expression is no tissue-specific and this protein was proposed to protect cells against oxidative stress. To understand the impact of the disulfide bond on structural dynamics in Ngb and Cygb, we determined kinetics and thermodynamics for CO photo-release and rebinding to both proteins in the presence and absence of the disulfide bond (Ngb^{red} and $Cygb^{red}$). Photoacoustic calorimetry (PAC) data indicate that CO photo-dissociation from Ngb and Ngb^{red} is monophasic (τ<50 ns) leading to an endothermic enthalpy change (ΔH~20 kcal mol^{-1}) and the volume change is ~3 times larger for hNgb than $hNgb^{red}$ (ΔV=4.4 ± 0.3 mL mol^{-1}). Interestingly, biphasic kinetics were observed for CO migration from the heme pocket of Cygb with τ_1<50 ns and an additional τ_2~200 ns at 20 ^oC. $Cygb^{red}$ produces monophasic kinetics with τ<50 ns suggesting that the disulfide bond strongly modulates the ligand migration pathway. CO photo-release from Cygb first leads to a volume expansion ($\Phi\Delta V_1$= 4.2 ± 1.9 mL mol^{-1}) and endothermic enthalpy change ($\Phi\Delta H_1$= 23.3 ± 9.1 kcal mol^{-1}), followed by a smaller volume expansion ($\Phi\Delta V_2$= 3.7 ± 1.5 mL mol^{-1}) and exothermic enthalpy change ($\Phi\Delta H_2$= −11.8 ± 7.0 kcal mol^{-1}). Upon disruption of the disulfide bond, CO photo-dissociation from $Cygb^{red}$ produces a prompt endothermic enthalpy change ($\Phi\Delta H$= 6.3 ± 0.4 kcal mol^{-1}) and a small volume expansion ($\Phi\Delta V$= 1.8 ± 0.8 mL mol^{-1}). These results suggest different mechanisms of ligand migration among vertebrate hexacoordinate hemoglobins as well as a larger impact of the disulfide bond on mechanism of ligand migration in Cygb than in Ngb.

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Cytochrome C_6 of *Chlamydomonas Reinhardtii* - A Heme Protein with Unusual Properties

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Cytochrome $c_6$is a lumenal redox carrier functioning on the oxidizing side of Photosystrem I in oxygenic photosynthesis. We have constructed a synthetic gene, expressed, purified, and conducted an initial characterization for the cytochrome c_6 from the green alga, *Chlamydomonas reinhardtii*. The synthetic gene was constructed by the removal introns and the substitution of codons for those best suited for expression in *E. coli*. The gene was incorporated into a plasmid downstream of the lac operon and a pelA leader sequence, which facilitates the exportation of the protein to the periplasm. The protein is expressed by a cotransformation in E. coli with the wild-type plasmid and the PEC86 plasmid, which contains a set of genes for the covalent attachment of the heme to the protein and a gene for chloramphenicol resistance. Although the expressed protein showed many characteristics (UV-visible spectra, redox potential, etc) typical of c-type cytochromes, it also exhibited some unusual properties. (i) Although the redox potential was as expected for cytochrome c6 at pH 7.0, the redox potential did not exhibit a pH dependent transition at high pH. (ii) The CD spectrum of oxidized cytochrome c6 lacked a negative peak in the Soret region of the spectrum in contrast to most c-type cytochromes. (iii) The thermal stability as measured by DSC was significantly higher (Tm = 78 C) than for most c-type cytochromes (Tm = 50-60 C). Possible explanations for the structural bases for these unusual properties will be discussed.

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Micro-Raman Spectroscopy of the Hemoglobin Iron-Histidine Bond in Single Erythrocytes

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The iron-histidine vibrational mode probes the crucial iron-protein linkage in heme proteins, and it has been established as a sensitive structural probe of the proximal heme pocket from studies of proteins in solution. The cellular environment can modify the interactions and dynamics from the isolated protein. We present Raman measurements on individual erythrocytes under physiological conditions over the frequency range from 150 to 1700 cm-1. Raman spectra were excited with the 442 nm line from a He-Cd laser using 2-3 mW power at the sample. Photodissociation of the ligated hemoglobin is achieved by the